BB206 Tutorial:

RESTRICTION MAPPING

Let us start simply:

Example 1. The linear DNA fragment shown here has cleavage sites for BamHI and EcoRI. In the accompanying diagram of an electrophoresis gel, indicate the positions at which bands would be found after digestion with:

a. BamHI alone
b. EcoRI alone
c. BamHI and EcoRI together
The dashed lines on the right indicate the positions which bands of 1 - 12 kb would migrate.
A. This is what the gel would look like if you digested this fragment with these enzymes

Now see if you can take this data, and work out the restriction map of the fragment

Now try another one.
Example 2. The circular DNA molecule shown below has cleavage sites for BamHI and EcoRI. In the accompanying diagram of an electrophoresis gel, indicate the positions at which bands would be found after
digestion with:
   a. *BamHI* alone
   b. *EcoRI* alone
   c. *BamHI* and *EcoRI* together.

The dashed lines on the right indicate the positions which
bands of 1 - 12 kb would migrate.

A. This is what the gel would look like if you digested this circular DNA plasmid with these enzymes

Now let us try to work out the restriction map of the plasmid just from the data we have here.

First, look at the gel, and determine the size fragments that are present in each lane.
I have put them in the following table
<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>Fragment Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI</td>
<td>7 kb, 3 kb</td>
</tr>
<tr>
<td>EcoRI</td>
<td>7 kb, 3 kb</td>
</tr>
<tr>
<td>EcoRI + BamHI</td>
<td>3 kb, 2 kb</td>
</tr>
</tbody>
</table>

Now, use the following steps as a guide to help you:

To generate a restriction map of a circular DNA molecule, try the following steps:

1. Draw a circle

2. Calculate the size:
   a. Add up the fragments from each of the different restriction digests - each should add up to the same number
   b. Remember, fragments of the same size will migrate the same distance and are not distinguishable if in the same lane

   \[
   \begin{align*}
   \text{EcoRI: } & 7 \text{ kb} + 3 \text{ kb} = 10 \text{ kb} \\
   \text{BamHI: } & 7 \text{ kb} + 3 \text{ kb} = 10 \text{ kb} \\
   \text{EcoRI} + \text{BamHI: } & 3\text{ kb} + 2\text{ kb} = 5 \text{ kb} \quad ???
   \end{align*}
   \]

   This is not the same as the first two, so how can we 'fit it'? The only logical explanation is that there are more than one fragments of the same size! In this case, there must be two 3 kb fragments and two 2 kb fragments

   \[
   3 \text{ kb} + 2 \text{ kb} + 3 \text{ kb} + 2 \text{ kb} = 10 \text{ kb}
   \]

   (Are there any other possibilities?)
3. Put in the first site

you may have noticed that this is arbitrary - YOU have to decide where to put it.

Here is a hint: by convention if there is one (or more) EcoRI site(s), one of these is designated as the first site and placed at 12 o'clock

4. Put in the remaining EcoRI sites (or whatever sites of the enzyme you have started with)

From the gel digest, there are two fragments (7 kb and 3 kb). This means there are two sites.
5. Put in sites for a second enzyme, and distance these relative to the EcoRI sites as well as to each other

We know that the second enzyme, BamHI, generates two fragments as well: 7 kb and 3 kb
So there must be 2 BamHI sites in the plasmid

We also know that the combination of BamHI and EcoRI generates fragments of 3 kb and 2 kb

Ask yourself the following questions, relative to the data:

Could any fragments from the EcoRI digest remain in the double digest with BamHI?
The answer in this case is YES - a 3 kb fragment is present in both.

Now ask, is it possible that both BamHI sites could be present in the remaining (7 kb) EcoRI fragment?
Again, the answer is YES - add it up if you don’t believe me

Try putting in the first BamHI site, and reorient it as you need to make sure the numbers add up, then add the remaining BamHI site. Again, make sure the numbers add up. If they don’t, try a different position.
This is what you should have drawn:

Helpful hint: Just keep adding up the numbers and make sure they add up to the same (the size you have calculated in step 2!)

Try this one:
Example 3. A 8.9 kb circular plasmid is digested with
three restriction enzymes, *EcoRI*, *BamHI* and *HindIII*, individually and in combination, and the resulting fragment sizes are determined by means of electrophoresis. The results are as follows:

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Fragment Sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td>8.9 kb</td>
</tr>
<tr>
<td>BamHI</td>
<td>6 kb, 2.9 kb</td>
</tr>
<tr>
<td>HindIII</td>
<td>8.9 kb</td>
</tr>
<tr>
<td>EcoRI + BamHI</td>
<td>6 kb, 2.4 kb, 0.5 kb</td>
</tr>
<tr>
<td>EcoRI + HindIII</td>
<td>7.4 kb, 1.5 kb</td>
</tr>
<tr>
<td>BamHI + HindIII</td>
<td>5 kb, 2.9 kb, 1 kb</td>
</tr>
<tr>
<td>EcoRI + BamHI + HindIII</td>
<td>5 kb, 2.4 kb, 1 kb, 0.5 kb</td>
</tr>
</tbody>
</table>

Draw a restriction map based on these results.  
A.
If you look carefully, you will see that these maps are MIRROR IMAGES of each other. Either would be correct because they both fit the data given in the table above (Can you think of the reason WHY there would be 2 correct answers?). The important point is that you would be able to PREDICT the fragment sizes correctly from
either map.

Remember - maps are a means of orientating yourself. But they can be drawn from different perspectives.

And it is important to be able to draw maps, because they help us PREDICT fragment sizes from restriction enzyme digests, which we can then VERIFY by performing the digest of the actual DNA and running it on a gel. If you are cloning and manipulating DNA, this is an invaluable tool to help you identify and distinguish between different DNA fragments, find specific DNA fragments (and may even tell you whether you have got the right or the WRONG clone!)

If you are feeling more adventurous, try the sample problem solving test from a previous year.

This tutorial was designed by Dr Eve Lutz. Should you need more help or information, please contact her (email eve.lutz@strath.ac.uk).

For more background information on restriction enzymes and restriction mapping, please click on the links to the BB211 class webpages Restriction enzymes or Restriction mapping or go to the BB211 classpage on Bruce.